THE INFRARED ABSORPTION SPECTRA OF PROTEINS IN THE 3 μ REGION

BY

KARL FREDERICK KREBS

B.S., University of Illinois, 1936

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1940

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The author wishes to express his sincere appreciation to Professor A. M. Buswell whose advice and direction made this investigation possible; also to Professor W. H. Rodebush, Dr. R. C. Gore and Dr. F. T. Wall for helpful criticism and interpretation of certain phases of the problem, and finally to the Rockefeller Foundation for financial support for this research.

Introduction

The study of bound water in biological tissues has been handicapped from its inception by the weaknesses inherent in the methods used for the determination of bound water. None of the various methods give any clue as to the mechanism of bonding, and all are subject to criticism on the basis of the fundamental assumptions involved.[1]

A possible mechanism for the bonding of water was suggested by Buswell in 1928.[2] His suggestion that water was bonded to gelatin by means of the hydrogen-bond followed from correlation of the paper by Latimer and Rodebush[3] who suggested the hydrogen-bond hypothesis, and the classic work in spectroscopy of Coblentz[4] who had noticed peculiarities in the infrared absorption of water at 1.5 mu when present as "water of constitution" in certain minerals and in a gelatin film.

The work of Wulf and Liddel[5] who showed that the first harmonic of the OH group disappeared when opportunities for hydrogen bonding were present indicated that the infrared absorption technique offered a method of testing this proposed mechanism, and also gave promise of being adaptable to quantitative determination of bound water in biological hydrogels. However, due to the complexity of the NH and OH absorption spectra in the bonded state, it has been necessary to determine the various bonding frequencies of these groups in simple organic molecules[6] and also to work out their absorption spectra when present in such complicated structures as the proteins. This present work is then a report of the infrared absorption of a series which includes members of several of the classes of proteins based upon solubilities.

Experimental

The spectrometer used was a prism type built in this laboratory in 1936. The source of radiation was a street lamp bulb which was supplied with a current of seven amperes from the 110 D.C. line balanced against six two-volt storage batteries to avoid line fluctuations. A more detailed discussion of the instrument is available in a previous publication.[7]

The materials studied were all too insoluble in suitable solvents

to permit study in the solution form, and so a method for obtaining them in the form of a thin film was devised. The solution of the protein was evaporated on a 24x50 mm. microscope cover slide floating on mercury. 0.5 cc. of a 1% solution of the proten in water, 0.6% NH_4OH, or 70% alcohol depending upon the properties of the protein, gave a film of the desired thickness.

Films built up by the Langmuir technique[8] were also tried, but were abandoned in favor of the simpler deposition method when no appreciable qualitative differences in the two types of film were found. The method would have a distinct advantage in quantitative determinations in that the molecular absorption coefficient could be calculated from the known number of layers. The preparation of such films would be laborious since they would have to be about 5000 layers in thickness.

In the hydration studies on two of the proteins a cell provided with air ducts to permit the passage of air across the film was used. This arrangement made it possible to regulate the degree of hydration of the film without removing it from the absorption cell. Different degrees of hydration were affected by passing the air through saturated salt solutions of known vapor pressure.

Results

The absorption bands found in the sixteen proteins studied are given in the following table together with the data obtained in the study of certain other related compounds.

Compound	Absorption Peaks (Wave Lengths in mu)					
Peptone	2.91	3.00		3.22	3.33	
Gelatin		2.97		3.22	3.34	
Rennet		2.97		3.20	3.38	
Zein		2.99		3.21	3.35	
Gliadin		2.99		3.21	3.34	
Hemoglobin		2.99		3.22	3.35	
Insulin		3.00		3.22	3.35	
Hordein		3.00		3.21	3.35	
Pepsin		3.01		3.22	3.35	
Salmine		3.01	3.09	3.20	3.35	
Glutenin		3.01		3.22	3.36	
Edestin		3.01		3.20	3.34	
Soybean Protein		3.02		3.20	3.35	
Ficin		3.02		3.22	3.34	
Casein		3.02		3.22	3.34	
Egg Albumin		3.03		3.22	3.34	
Glucose		2.96			3.40	
Agar		2.92			3.35	
β-amylose		2.96			3.39	
Glycyl-dl-alanine	2.85		3.09	3.22	3.36	
α-bromoisocarpoyl-leucine	2.79	3.03		3.23	3.35	
Alanine				3.23	3.40	3.48
Proline		2.97	3.14		3.34	
Urea	2.91	2.99	3.04			
N-butylurea		2.97	3.04		3.36	3.42
Thiourea	2.96	3.03	3.12			
N-phenyl-N', N'-dibutyl-thiourea		3.05		3.24	3.36	3.43

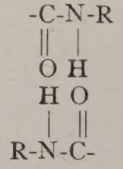
Discussion

(A) Proteins

The interpretation of the absorption peaks found in the proteins is based upon considerable experimental data obtained from the study of simple organic nitrogen compounds by Mr. J. R. Downing of this laboratory.[6]

While no final conclusions can be formulated at present, the most

logical interpretation of the data obtained to date has lead to the assignment of NH...N bonding at 3.00 mu; NH...O in a linear type of bonding such as has been shown to occur in glycine by X-ray data,[9] at 3.14 mu; and NH...O in a ring-dimer structure involving two -CO-NH-R groups at 3.22 mu. The ring-dimer association may be pictured as follows:



The appearance of the 3.22 and 3.00 mu bands in all of the proteins studied suggests the important role that the hydrogen bond plays in the structure of the proteins. These data afford experimental justification to the postulates on protein structures involving hydrogen bonds which have been made by several authors.[10]

The spiralled beta-keratin structure suggested by Neurath[11] appeals to the author as being highly probable in that it would appear to permit a closer approach of adjacent polypeptid chains thus allowing hydrogen bonding of the ring-dimer type between the peptid links. This type of linkage would explain the appearance of both the 3.22 and 3.00 mu bands. The spiralled structure might be compared to two twisted threads with the peptid links as the points of contact.

Salmine is a member of the protamine class of proteins which yield on hydrolysis comparatively few amino acids, among which the basic ones predominate. Salmine, obtained from salmon sperm, contains 87% arginine, $NH_2.CNH.NH.CH_2CH_2.CH_2.CHNH_2.COOH$, which has, in addition to the usual amino group, three extra nitrogen groups one of which is an imino group. The typical protein, on the other hand, is made up of many different amino acids. Only a very small per cent of these amino acids contain NH groups other than the amino group.

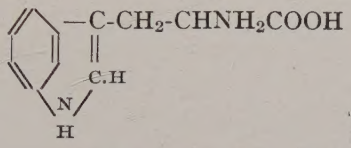
One would expect, therefore, that salmine would show an absorption spectra different from that obtained from most proteins. The band at 3.09 mu appearing in the absorption spectra for salmine is unique in the protein spectra so far obtained. It undoubtedly arises as a result of the large excess of nitrogen hydrogen groups in the protein. It is not definitely determined whether this absorption is due to an

NH...O bonding or to a special type of NH...N bonding which involves the imino group.

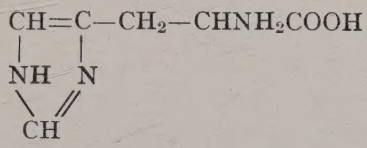
The need for further study of this protein and of simpler compounds containing the imino linkage is indicated. It is quite probable that very interesting structures could be postulated for salmine if more exact data as to type of bonding were available.

One is naturally concerned with the possibility of non-peptid-nitrogen bonds in other proteins. In going through the structures of the amino acids which have been found in the proteins one finds five amino acids containing non-peptid-nitrogens:

Tryptophane,



Histidine,



Lysine, $NH_2-CH_2-CH_2-CH_2-CH_2-CHNH_2-COOH$

Arginine, $NH_2-CNH-NH-CH_2-CH_2-CH_2-CHNH_2\ COOH$

Citrulline, $NH_2-CO-NH-CH_2-CH_2-CH_2-CHNH_2-COOH$

Of these amino acids tryptophane is rarely present in amounts greater than 3%, histidine is also low in most proteins, lysine varies from 0 to 8% in the proteins considered here, the presence of citrulline in most proteins has not yet been verified, and arginine varies from 1 to 15% in the usual protein.

The characteristics of the spectrum of salmine should be apparent in the spectrum of thymus histone which contains 27% arginine, 9.7% lysine and 5.8% histidine.[12]

It can be seen that the hydrogen bondings due to the presence of these amino acids in the ordinary protein would be only a very small contribution to the total bonding due to the peptid linkage. Since the amino acids are probably arranged in some sort of periodic arrangement in the proteins,[13] it would not be logical to assume that very many of

these extra NH groups would be close enough together in the peptid chain to permit bonding. The absorption of the free N-H groups in the protein would resemble that of the primary and secondary aliphatic amines. This type of N-H absorption has been shown to be very weak in intensity,[14] and therefore the presence of these few free amino groups, eg. lysine, might very well be missd in th spctra of the proteins. The effect of the small amounts of histidine and tryptophane occurring in certain proteins cannot be detected in the absorption spectra with the present methods. One would expect therefore that there would be a great similarity in the absorption spectra in this region.

Peptone also shows a peculiarity in the infrared absorption in that it has a distinct shoulder on the shore wave side of the 3.00 mu band. This peak, if separated from the 3.00 mu band, would occur a little below 2.9 mu. It appears likely that the band represents unbonded NH groups in the protein fragments that make up the peptone. This might be expected in such a mixture of components since there would be lack of orientation and complete randomness of arrangement.

So far in this discussion no mention has been made of differences appearing in the CH absorption of the proteins due to differences in the side chain residues. The reason for this is that the CH band is not very prominent, and no significant observations can be made either as to prominence or exact location of the absorption maxima. The only differences detectable would be in the relative amounts of aliphatic and aromatic carbon-hydrogen, but the relative percentages of those amino acids having aromatic rings is fairly constant in the proteins and the percentage is also relatively low.

Bonding between hydroxel and carboxyl groups in proteins is possible, but resolving power available does not permit their observation. Their number would be small in comparison to bonds involving nitrogen.

Structural linkages between chains other than the hydrogen bond are not detectable in the 3 mu region. Of these proposed linkages the disulfide might be expected to appear at a wave length greater than 10 mu, and the ester or salt linkages between acidic and basic amino acids might be detected in the carbonyl region at 5.8 mu.

(B) Hydration Studies

The bonded frequency for water-like hydroxyl groups has been shown to appear at a wave length slightly lower than 3 mu.[15] The

results from the study of the carbohydrates support these data in that the bonded frequency occurs at 2.96 mu. The superposition of the absorption due to structural hydrogen-bonding and of the absorption of bonded water hydroxyls make hydration studies on the carbohydrates impracticable in the 3 mu region. In the proteins these two types of bonding are separated to some extent since the predominate type of structural hydrogen-bonding involves the NH groups which show absorption in the bonded state at slightly longer wave length than the hydroxyl groups do. The shoulder that appears on the short wave side of the 3 mu band gives a qualitative picture of water bonding, but the separation is not great enough for any quantitative measurements with the present technique and instrument. It is hoped that improvements in the weighing technique and the use of an instrument of greater resolving power will enable a quantitative determination to be made in the future.

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VITA

Karl Frederick Krebs was born in Oxford, Wisconsin, on November 21, 1914. He attended grammar school in Newton and Greenville, Illinois. From 1928 to 1932 he attended the Greenville High School. He was awarded a County Scholarship to the University of Illinois in June 1932, and he has attended this university for his entire advanced education. After receiving the degree of Bachelor of Science in 1936, he continued graduate work in physical and biochemistry. Since June 1936 he has held a special research assistantship in physical chemistry made possible through the Rockefeller Foundation. He is a member of Phi Beta Kappa, Phi Kappa Phi, Sigma Xi, and Phi Lambda Upsilon.